PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 07 August 2000 (07.08.00)	
International application No. PCT/GB99/03936	**Applicant's or agent's file reference** IT/SC/N8976
International filing date (day/month/year) 25 November 1999 (25.11.99)	**Priority date** (day/month/year) 26 November 1998 (26.11.98)
Applicant EBRINGER, Alan	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

21 June 2000 (21.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Juan Cruz
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

GB9903936

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

TOLLETT, Ian
WILLIAMS, POWELL & ASSOCIATES
4 St.Paul's Churchyard
LONDON EC4M 8AY
GRANDE BRETAGNE

Williams Powell & Associates
RECEIVED
23 NOV 2000

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	20.11.2000
Applicant's or agent's file reference IT/N8976	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/03936	25/11/1999	26/11/1998

Applicant
KING'S COLLEGE et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Danti, B Tel.+49 89 2399-8161

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
IT/N8976		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/03936	25/11/1999	26/11/1998
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant KING'S COLLEGE et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/06/2000	20.11.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Tilkorn, A-C Telephone No. +49 89 2399 8688

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/03936

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-5	as received on	23/10/2000	with letter of	18/10/2000

Claims, No.:

1-10	as received on	23/10/2000	with letter of	18/10/2000

Drawings, sheets:

1/4-4/4	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-7,9,10
	No:	Claims 8
Inventive step (IS)	Yes:	Claims 5,9
	No:	Claims 1-4,6-8,10
Industrial applicability (IA)	Yes:	Claims 1-10
	No:	Claims -

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sh et

Re It m I

The amendments carried out on **claims 1, 3** and **8** and the corresponding parts of the description contravene Art 34(2)(b) PCT, because they go beyond the disclosure as originally filed. The replacement of the term "myelin neurofilaments" with "neurofilaments" is not supported by the original application.
Neurofilaments as described in standard textbooks such as "Molecular Biology of the Cell" (2. edition, Alberts et al.; Garlan Publishing Inc., 1989; p 663 para 2) consist of three neurofilament proteins none of which being myelin.
Myelin sheaths however are only formed by specialized Schwann cells ("Molecular Biology of the Cell" (2. edition, Alberts et al.; Garlan Publishing Inc., 1989; p 1073 para 2)
Hence, myelin neurofilaments are specific neurofilaments and consequently, antibodies which bind to neurofilaments do not necessarily bind to myelin neurofilaments.
Although the original application contains an example in which antibodies binding to bovine neurofilaments are assayed, it does not form a basis for a more general claim directed to a method involving testing for antibodies which bind to "neurofilaments".
Thus, this report is established as if said amendments had not been made.
A copy of the above cited literature is appended to this report.

Re Item V

The following documents are referred to in this communication:

D1: ENVIRONMENTAL HEALTH PERSPECTIVES, vol. 105, no. 11, November 1997 (1997-11), pages 1172-1174
D2: WO 98 13694 A (2 April 1998)
D3: WO 98 03647 A
D4: WO 99 47932 A (23 September 1999)

1 Novelty (Art 33(2) PCT):

1.1 The subject-matter of **claim 1** embraces embodiments that involve assaying a biological sample for antibodies which bind to myelin or to one or more antigenic (immunogenic) parts thereof.

Claim 1 is novel because none of the available documents discloses a diagnostic method that involves the detection of antibodies directed to myelin or antigenic parts thereof neither an assay testing a biological sample for antibodies which bind to myelin neurofilaments or to one or more antigenic fragments thereof. Consequently, dependent **claims 2-7** are also novel.

1.2 **Claim 8** is directed to a kit comprising myelin, myelin neurofilaments or parts thereof. Thus, a kit which has as single constituent myelin falls within the scope of the claim. Recombinant myelin proteins are described in the art (e.g. D3: p 2 l 30 - p 3 l 17). As a myelin preparation as described in the art cannot be distinguished from the kit as claimed, the subject-matter of claim 8 is not novel.

1.3 it extends to kit is not novel, because myelin protein and fragments thereof are known in the art (e.g. D3: p 2 l 30 - p 3 l 5). A kit as such is not considered to be a technical feature. Thus, a kit merely comprising myelin is not novel.

1.4 **Claims 9** and **10** are novel, because the specific peptide sequences (SEQ ID Nos 1-8) and a kit containing myelin and Acinetobacter calcoaceticus have previously not been disclosed.

2 Inventive Step (Art 33(3) PCT):

2.1 **Claim 1** does not appear to satisfy Art 33(3) PCT for the following reasons:
The underlying hypothesis of the present application is that spongiform disease is caused by cross-reactive autoantibodies evoked following exposure to biological material containing certain bacteria e.g. Acinetobacter sp. (application: p 1 l 12-17). In other words, Acinetobacter sp. exhibits molecular mimicry of antigens of the nervous system (e.g. myelin or myelin fragments) of mammals. Cattle exposed to Acinetobacter thus produce antibodies that bind with Acinetobacter antigens but also with self antigens. This hypothesis is known from each of the documents D1 (p 1173 col 1 para 3) and D2 (abstract, p 2 last paragraph- p 3 para 1).
On the basis of the above hypothesis the antibodies detected according to the method of present claim 1 should also bind to an antigenic peptide that exhibits molecular mimicry of a mammalian myelin peptide. Thus, the diagnostic test of D2

(D2: p 2 last paragraph) detects the same antibodies as the diagnostic test of present claim 1 but the assayed antigens are different. It is stated in D2, that the detected antibodies cross-react with myelin (D2: p 2 last paragraph).
Since the underlying hypothesis is known from D2, which is considered to represent the closest prior art, it appears to be an arbitrary choice to assay either antibodies which bind to antigenic peptides of Acinetobacter that exhibit molecular mimicry of a mammalian myelin peptide or to assay antibodies which bind to myelin or antigenic parts thereof (Guidelines IV 8.8 C1). The provision of the test antigen, namely myelin or myelin fragments, is well known in the art (see e.g. D3: p 2 l 30 - p 3 l 5). Since prima facie the antigen myelin is more easily available than the antigenic peptides of Acinetobacter, it appears obvious for the skilled person to choose the more convenient antigen i.e. myelin in order to set up a diagnostic method.
Furthermore, the formulation of claim 1 does not require that the test has to be carried out for both antigens: myelin and myelin neurofilaments, which is described in one embodiment (appl.: p 4 l 24-25). According to the wording of claim 1 the scope of the claim includes methods, which only test for antibodies which bind to myelin and/or antigenic parts thereof.
In conclusion, inventiveness cannot be acknowledged for **claim 1**. The same arguments apply to the dependent **claims 2-4,6** and **7** as these claims do not contain an inventive concept per se. Similarly, the testkit according to **claim 10** does not appear to involve inventive activity.

2.2 The method of **claim 5** and the diagnostic kit of **claim 9** appear to satisfy Art 33(3) PCT because none of the available prior art documents renders the selection of the peptides identified by SEQ ID NOs 1-8 obvious.

Re Item VI

Since the priority of the present application is valid, D4, which was published (23.9.99) after the priority date (26.11.98) of the present application does not belong to the state of the art. However, in some cases D4 might become relevant in the regional phase for the assessment of novelty (e.g. before the EPO pursuant to Art 54(3) EPC).

Re Item VII

The expression "incorporated herein by reference" in respect of prior art documents (e.g. page 3 l 16) leads to a doubt as to whether the requirement of the description being self-contained is satisfied (Guidelines II, 4.17).

Re Item VIII

Claim 1 does not satisfy Art 6 PCT because the expression "biological sample" is too broad and therefore not fully supported by the description. "Biological sample" comprises for example a urine sample, but there is no indication throughout the application that a sample other than a serum sample (p 4 l 1 and l 20-22) is suitable the for claimed diagnostic method.

DIAGNOSIS OF DEMYELINATING OR SPONGIFORM DISEASE

This invention relates to the diagnosis of demyelinating diseases and spongiform encephalopathies in animals and humans, especially BSE and similar or related diseases in humans.

In our co-pending International application WO97/02667 we have disclosed a new diagnostic test for spongiform encephalopathy and other demyelinating conditions in mammals. The test disclosed in our prior application is based on a model of the genesis of this pathological state which is applicable to the various forms in which it is manifest in humans and other animals. In relation to the bovine spongiform disease this model provides an alternative to the current theory based on the formation of prions. Briefly, the new model is based on the phenomenon of molecular mimicry according to which mammals exposed to certain bacteria having peptide sequences which mimic myelin peptides experience an auto-immune reaction. Foremost among the bacteria that are involved in the induction of the auto-immune reaction are Acinetobacter species, especially Acinetobacter calcoaceticus. The diagnostic test based on the new model opens up the possibility of early treatment of these infections e.g. by use of an appropriate antibiotic to prevent further auto-immune attack on the animal's own myelin.

In our International application WO99/47932, we have confirmed the presence of elevated levels of Acinetobacter IgA antibodies in sera of patients suffering from multiple sclerosis (MS) and Creutzfeld-Jacob disease CJD.

In our priority UK application 9825948.4 we described further tests which confirmed the presence of antibodies to bovine myelin and also to bovine neurofilaments in the sera of cows that have died from BSE. These antibodies are of the IgA type. Similar results have also been obtained with sera from patients suffering from MS and CJD. These findings confirm the validity of the model described above and permit the conclusion that we have discovered a general pattern of the origin of similar diseases that occur or may

occur in vertebrates including humans and other farm animals e.g. in poultry farms. Our latest results also provide the basis of a further test for the early identification of these diseases, especially incipient BSE in cows. This further test may either be alternative to or additional to that based on the detection of IgA antibodies to Acinetobacter species e.g. Acinetobacter calcoaceticus.

The present invention therefore comprises a method for diagnosing spongiform disease or demyelinating disease in vertebrates, including BSE, MS and CJD, which comprises assaying a biological sample for antibodies, especially IgA antibodies, which bind to myelin and/or neurofilaments or antigenic (immunogenic) parts thereof, including peptide components as hereinafter specified.

The method preferably comprises assaying for antibodies to myelin and /or neurofilaments of vertebrate species e.g bovine or human species. However, myelin and neurofilaments from other species which are sufficiently homologous to those of bovine or human species to bind to the antibodies under estimation may alternatively be used .

In carrying out the method a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.

The invention also comprises a diagnostic kit for the detection of spongiform disease or demyelinating disease in vertebrates comprising, as test antigen, myelin or neurofilaments or antigenic (imunogenic)parts thereof.

The test antigen used in the above defined method and diagnostic kit may be a peptide component of the myelin or neurofilaments, such as one of the following peptides having Sequence ID Nos 1-8, namely,

1. NEALEK 2. LKKVHEE 3. EALEKQL 4. ELEDKQN
2. EALEKQL 6. KKVHEE 7. EIRDLR 8. EQEIRDLR

The above sequences have been retrieved from the Protein Information Resource database release 44.

In view of the greater specificity of the IgA antibodies in the immune response it may be concluded that the mechanism of infection with Acinetobacter is via the mucous membranes of the body, the primary sites being the gut or the nasal passages. It is possible that the nasal passages are the site of infection, resulting from inhalation of dust formed from dried sewage or animal excrement and carrying Acinetobacter. The knowledge of this mechanism implies the need for improved hygiene practices in the rearing of farm animals.

Experimental

Assays for the above mentioned organisms are described in our co-pending applications identified above, the contents of which are hereby incorporated by reference. Similar assay procedures using myelin protein or neurofilaments as test antigens are described below.

ELISA TEST:

(1) Aliquots of 200ul of the antigen suspension A or B were absorbed on 96 well flat bottomed rigid polystyrene microtitre plates overnight at 4 deg. Cent. (Antigen A is bovine myelin from Sigma Chemical Company, Fancy Road, Poole, Dorset, BH12 4XA, UK, at a concentration of 5ug/ml and antigen B is bovine neurofilaments from Sigma also at a concentration of 5ug/ml).

(2) The plates are then washed 3 times with phosphate buffered saline (PBS) 0.1% (v/v) Tween 20.

(3) Aliquots of 300ul of blocking solution (0.2% w/v ovalbumin, 0.1% v/v Tween) in PBS is added to each well and incubated for one hour at 37 deg. Cent.

(4) The plates are then washed 3 times with PBS. Tween 20.

(5) Aliquots of 200ul serum samples (test or control) diluted 1/200 in PBS. Tween is added and incubated for 2 hours at 37 deg. Cent.

(6) The plates are then washed 3 times with PBS. Tween 20.

(7) Aliquots of 200ul of peroxidase conjugated rabbit anti-cow IgA (alpha chain) diluted 1/4000 with PBS. Tween are added and incubated for 2 hours at 37 deg. Cent.

(8) The plates are then washed 3 times with PBS. Tween 20.

(9) The development of the colorimetric assay takes place at room temperature for 20 minutes, after the addition of 200ul per well of 0.5 mg/ml (2,2'-azinobis (3-ethylbenz-thiazoline-6-sulphonic acid) in citrate/ phosphate buffer, pH 4.1, containing 0.98 mM hydrogen peroxide.

(10) The reaction is then stopped with 100ul of 2 mg/ml sodium fluoride and optical densities measured at a wavelength of 630 nm with a micro-ELISA plate reader.

(11) All assays are done under coded conditions, in that the tester is unaware of the origin of the serum being studied (Test or control).

(12) All tests are done in duplicate.

The foregoing test procedure may be carried out in the same manner using human myelin or neurofilaments or peptides derived therefrom.

This assay is a novel way of diagnosing cattle suffering from bovine spongiform encephalopathy and humans suffering from MS and CJD in that it describes a test where antibodies to two brain antigens can be determined in bovine or human sera. Any reading in excess of 2 standard deviations of the healthy controls would indicate a positive response. Furthermore the test should be positive (above 2 standard deviations) for both antigens: (A) Bovine myelin protein and (B) Bovine neurofilaments.

This is the first assay that describes measurements of autoantibodies to brain antigens in BSE affected cattle and patients with MS and CJD.

Results for BSE are shown in the accompanying Figures 1 and 2.

Those for MS and CJD are shown in the accompanying Figure 3.

The tests described in our above-mentioned International applications may be combined with that of the present invention. This combined test is particularly suitable for use in testing for BSE. This combined test may be termed the "MAN test" and is based on separate measurements of autoantibodies to bovine myelin (white matter of the brain) and to bovine neurofilaments (gray matter of the brain), as well as to specific antibodies to the saprophytic bacterium Acinetobacter calcoaceticus.

The auto-antibodies to bovine myelin and to bovine neurofilaments and antibodies to Acinetobacter are measured as previously described, for each animal tested. The MAN index is then obtained by multiplying the optical densities according to the following algorithm: =

Myelin IgA autoantibody x Acinetobacter antibody x Neurofilaments autoantibody i.e. the multiplication product M x A x N.

The accompanying Figure 4 shows the results of this test when compared to healthy "organic"controls or to controls (CVL) suffering from other diseases. (CVL = Central Veterinary Laboratory, UK, from where these sera from animals with other diseases were obtained).

The MAN test is calibrated against "organic" farm controls, that is animals coming from a farm where the feedstuffs consist of grass and hay only. The MAN test is an empirical test, in that very low values are obtained for the MAN index, when healthy cows only are tested.

A positive response is recognised when the MAN index is 3 standard deviations above the value found in controls, when testing the serum of a cow suspected of having BSE.

CLAIMS

1. A method for diagnosing spongiform disease or demyelinating disease in vertebrates, including BSE, MS and CJD, which comprises assaying a biological sample for antibodies which bind to myelin and/or neurofilaments or to one or more antigenic (immunogenic) parts thereof.

2. A method according to claim 1, in which the antibodies are IgA antibodies.

3. A method according to claim 1 or 2, in which the assay is for antibodies that bind to vertebrate myelin and/or neurofilaments or parts thereof .

4. A method according to claim 3, in which the vertebrate is bovine or human.

5. A method according to claim 4, in which the test antigen is a peptide selected from the group consisting of peptides having sequences identified as Sequence ID Nos. 1 to 8 hereinbefore specified.

6. A method according to any of claims 1 to 5, in which a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.

7. A method according to any of the preceding claims combined with an assay for antibodies to Acinetobacter species.

8. A diagnostic kit for the detection of spongiform disease or demyelinating disease in vertebrates comprising, as test antigen, myelin and/or neurofilaments and/or one or more parts thereof.

9. A diagnostic kit according to claim 8, in which the test antigen is a peptide having a sequence selected from the group consisting of Sequence ID Nos. 1 to 8 specified hereinbefore.

10. A diagnostic kit according to claim 8 or 9, containing as test antigens myelin, neurofilaments, and *Acinetobacter calcoaceticus*.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
WILLIAMS, POWELL & ASSOCIATES
Attn. TOLLETT, Ian
4 St.Paul's Churchyard
LONDON EC4M 8AY
UNITED KINGDOM

Williams Powell & Assoc.
RECEIVED
10 APR 2000

Applicant's or agent's file reference IT/SC/N8976	Date of mailing (day/month/year) 05/04/2000 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/GB 99/ 03936	International filing date (day/month/year) 25/11/1999
Applicant KING'S COLLEGE et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41–22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL–2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Jaap Hurenkamp

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
IT/SC/N8976		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/03936	25/11/1999	26/11/1998
Applicant		
KING'S COLLEGE et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03936

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EBRINGER, A. ET AL.: "Bovine Spongiform Encephalopathy: Is It an Autoimmune Disease Due to Bacteria Showing Molecular Mimicry with Brain Antigens" ENVIRONMENTAL HEALTH PERSPECTIVES, vol. 105, no. 11, November 1997 (1997-11), pages 1172-1174, XP000892832	1,3,4,7, 8
A	table 1	2,5,9
X	WO 98 13694 A (EBRINGER ALAN ;KING S COLLEGE (GB)) 2 April 1998 (1998-04-02) cited in the application	1,3,4,7, 8
A	page 4, line 13 -page 5; claim 1	2,5,9

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 March 2000	05/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Gundlach, B

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A WAJGT: "Assessment by immunofluorescence methods of humoral antimyelin antibody in rats with cyanide encephalopathy" CHEMICAL ABSTRACTS + INDEXES,US,AMERICAN CHEMICAL SOCIETY. COLUMBUS, vol. 11, no. 80, 18 March 1974 (1974-03-18), page 68 XP002052988 ISSN: 0009-2258 abstract	1
P,X	WO 99 47932 A (EBRINGER ALAN ;KING S COLLEGE UNIVERSITY OF L (GB)) 23 September 1999 (1999-09-23)	1-4,6-8, 10
A	page 1-13	5,9

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/03936

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9813694 A	02-04-1998	EP 0929813 A	21-07-1999
WO 9947932 A	23-09-1999	AU 2948799 A	11-10-1999

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

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Applicant		
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[ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]B 99/03936

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 ·G01N33/68

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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A	table 1	2,5,9

X	WO 98 13694 A (EBRINGER ALAN ;KING S COLLEGE (GB)) 2 April 1998 (1998-04-02) cited in the application	1,3,4,7, 8
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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search	Date of mailing of the international search report
24 March 2000	05/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gundlach, B

1

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INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]GB 99/03936

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	A WAJGT: "Assessment by immunofluorescence methods of humoral antimyelin antibody in rats with cyanide encephalopathy" CHEMICAL ABSTRACTS + INDEXES,US,AMERICAN CHEMICAL SOCIETY. COLUMBUS, vol. 11, no. 80, 18 March 1974 (1974-03-18), page 68 XP002052988 ISSN: 0009-2258 abstract	1
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A	page 1-13	5,9

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/03936

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9813694 A	02-04-1998	EP 0929813 A	21-07-1999
WO 9947932 A	23-09-1999	AU 2948799 A	11-10-1999

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : G01N 33/68

A1

(11) International Publication Number: WO 00/31545

(43) International Publication Date: 2 June 2000 (02.06.00)

(21) International Application Number: PCT/GB99/03936

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(30) Priority Data:
9825948.4 26 November 1998 (26.11.98) GB

(71) Applicant *(for all designated States except US)*: KING'S COLLEGE [GB/GB]; Strand, London WC2R 2LS (GB)..

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: EBRINGER, Alan [AU/GB]; 76 Gordon Road, Ealing, London W5 2AR (GB).

(74) Agents: TOLLETT, Ian et al.; Williams, Powell & Associates, 4 St. Paul's Churchyard, London EC4M 8AY (GB).

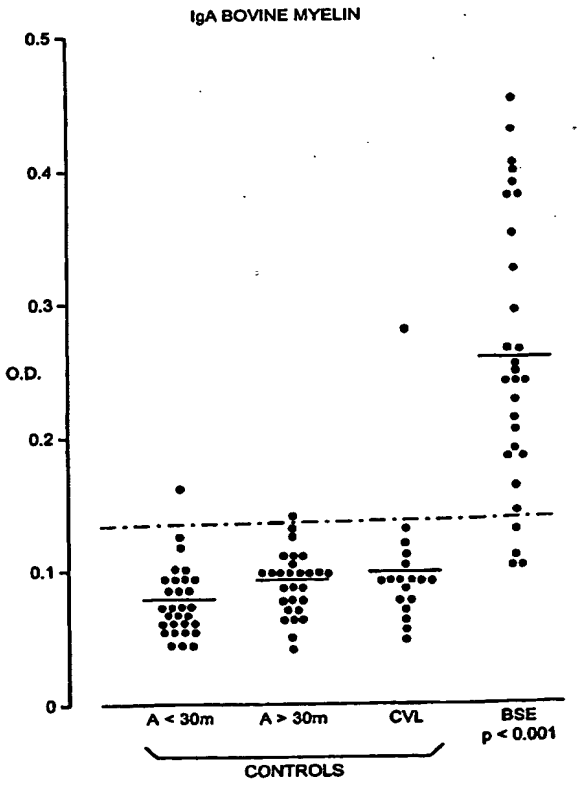
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: DIAGNOSIS OF DEMYELINATING OR SPONGIFORM DISEASE

(57) Abstract

A method for diagnosing spongiform disease or demyelinating disease in vertebrates, including BSE, MS and CJD, which comprises assaying a biological sample for antibodies which bind to myelin and/or myelin neurofilaments or to one or more antigenic (immunogenic) parts thereof.



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Diagnosis of Demyelinating or Spongiform Disease

This invention relates to the diagnosis of demyelinating diseases and spongiform encephalopathies in animals and humans, especially BSE and similar or related diseases in humans.

In our co-pending International application WO97/02667 we have disclosed a new diagnostic test for spongiform encephalopathy and other demyelinating conditions in mammals. The test disclosed in our prior application is based on a model of the genesis of this pathological state which is applicable to the various forms in which it is manifest in humans and other animals. In relation to the bovine spongiform disease this model provides an alternative to the current theory based on the formation of prions. Briefly, the new model is based on the phenomenon of molecular mimicry according to which mammals exposed to certain bacteria having peptide sequences which mimic myelin peptides experience an auto-immune reaction. Foremost among the bacteria that are involved in the induction of the auto-immune reaction are Acinetobacter species, especially Acinetobacter calcoaceticus. The diagnostic test based on the new model opens up the possibility of early treatment of these infections e.g. by use of an appropriate antibiotic to prevent further auto-immune attack on the animal's own myelin.

In our International application WO99/47932, we have confirmed the presence of elevated levels of Acinetobacter IgA antibodies in sera of patients suffering from multiple sclerosis (MS) and Creutzfeld-Jacob disease CJD.

In our priority UK application 9825948.4 we described further tests which confirmed the presence of antibodies to bovine myelin and also to bovine myelin neurofilaments in the sera of cows that have died from BSE. These antibodies are of the IgA type. Similar results have also been obtained with sera from patients suffering from MS and CJD. These findings confirm the validity of the model described above and permit the conclusion that we have discovered a general pattern of the origin of similar diseases that

occur or may occur in vertebrates including humans and other farm animals e.g. in poultry farms. Our latest results also provide the basis of a further test for the early identification of these diseases, especially incipient BSE in cows. This further test may either be alternative to or additional to that based on the detection of IgA antibodies to Acinetobacter species e.g. Acinetobacter calcoaceticus.

The present invention therefore comprises a method for diagnosing spongiform disease or demyelinating disease in vertebrates, including BSE, MS and CJD, which comprises assaying a biological sample for antibodies, especially IgA antibodies, which bind to myelin and/or myelin neurofilaments or antigenic (immunogenic) parts thereof, including peptide components as hereinafter specified.

The method preferably comprises assaying for antibodies to myelin and /or myelin neurofilaments of vertebrate species e.g bovine or human species. However, myelin and myelin neurofilaments from other species which are sufficiently homologous to those of bovine or human species to bind to the antibodies under estimation may alternatively be used .

In carrying out the method a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.

The invention also comprises a diagnostic kit for the detection of spongiform disease or demyelinating disease in vertebrates comprising, as test antigen, myelin or myelin neurofilaments or antigenic (imunogenic)parts thereof.

The test antigen used in the above defined method and diagnostic kit may be a peptide component of the myelin or myelin neurofilaments, such as one of the following peptides having Sequence ID Nos 1-8, namely,

1. NEALEK 2. LKKVHEE 3. EALEKQL 4. ELEDKQN

2. EALEKQL 6. KKVHEE 7. EIRDLR 8. EQEIRDLR

The above sequences have been retrieved from the Protein Information Resource database release 44.

In view of the greater specificity of the IgA antibodies in the immune response it may be concluded that the mechanism of infection with Acinetobacter is via the mucous membranes of the body, the primary sites being the gut or the nasal passages. It is possible that the nasal passages are the site of infection, resulting from inhalation of dust formed from dried sewage or animal excrement and carrying Acinetobacter. The knowledge of this mechanism implies the need for improved hygiene practices in the rearing of farm animals.

Experimental

Assays for the above mentioned organisms are described in our co-pending applications identified above, the contents of which are hereby incorporated by reference. Similar assay procedures using myelin protein or neurofilaments thereof as test antigens are described below.

ELISA TEST:

(1) Aliquots of 200ul of the antigen suspension A or B were absorbed on 96 well flat bottomed rigid polystyrene microtitre plates overnight at 4 deg. Cent. (Antigen A is bovine myelin from Sigma Chemical Company, Fancy Road, Poole, Dorset, BH12 4XA, UK, at a concentration of 5ug/ml and antigen B is bovine neurofilaments from Sigma also at a concentration of 5ug/ml).

(2) The plates are then washed 3 times with phosphate buffered saline (PBS) 0.1% (v/v) Tween 20.

(3) Aliquots of 300ul of blocking solution (0.2% w/v ovalbumin, 0.1% v/v Tween) in PBS is added to each well and incubated for one hour at 37 deg. Cent.

(4) The plates are then washed 3 times with PBS. Tween 20.

(5) Aliquots of 200ul serum samples (test or control) diluted 1/200 in PBS. Tween is added and incubated for 2 hours at 37 deg. Cent.

(6) The plates are then washed 3 times with PBS. Tween 20.

(7) Aliquots of 200ul of peroxidase conjugated rabbit anti-cow IgA (alpha chain) diluted 1/4000 with PBS. Tween are added and incubated for 2 hours at 37 deg. Cent.

(8) The plates are then washed 3 times with PBS. Tween 20.

(9) The development of the colorimetric assay takes place at room temperature for 20 minutes, after the addition of 200ul per well of 0.5 mg/ml (2,2'-azinobis (3-ethylbenz-thiazoline-6-sulphonic acid) in citrate/ phosphate buffer, pH 4.1, containing 0.98 mM hydrogen peroxide.

(10) The reaction is then stopped with 100ul of 2 mg/ml sodium fluoride and optical densities measured at a wavelength of 630 nm with a micro-ELISA plate reader.

(11) All assays are done under coded conditions, in that the tester is unaware of the origin of the serum being studied (Test or control).

(12) All tests are done in duplicate.

The foregoing test procedure may be carried out in the same manner using human myelin or myelin neurofilaments or peptides derived therefrom.

This assay is a novel way of diagnosing cattle suffering from bovine spongiform encephalopathy and humans suffering from MS and CJD in that it describes a test where antibodies to two brain antigens can be determined in bovine or human sera. Any reading in excess of 2 standard deviations of the healthy controls would indicate a positive response. Furthermore the test should be positive (above 2 standard deviations) for both antigens: (A) Bovine myelin protein and (B) Bovine neurofilaments.

This is the first assay that describes measurements of autoantibodies to brain antigens in BSE affected cattle and patients with MS and CJD.

Results for BSE are shown in the accompanying Figures 1 and 2.

Those for MS and CJD are shown in the accompanying Figure 3.

The tests described in our above-mentioned International applications may be combined with that of the present invention. This combined test is particularly suitable for use in testing for BSE. This combined test may be termed the "MAN test" and is based on separate measurements of
autoantibodies to bovine myelin (white matter of the brain) and to bovine neurofilaments (gray matter of the brain), as well as to specific antibodies to the saprophytic bacterium Acinetobacter calcoaceticus.
The auto-antibodies to bovine myelin and to bovine neurofilaments and antibodies and to Acinetobacter are measured as previously described, for each animal tested.
The MAN index is then obtained by multiplying the optical densities according to the following algorithm: =

Myelin IgA autoantibody x Acinetobacter antibody x Neurofilaments autoantibody
i.e. the multiplication product M x A x N.

The accompanying Figure 4 shows the results of this test when compared to healthy "organic"controls or to controls (CVL) suffering from other diseases. (CVL = Central Veterinary Laboratory, UK, from where these sera from animals with other diseases were obtained).

The MAN test is calibrated against "organic" farm controls, that is animals coming from a farm where the feedstuffs consist of grass and hay only. The MAN test is an empirical test, in that very low values are obtained for the MAN index, when healthy cows only are tested.

A positive response is recognised when the MAN index is 3 standard deviations above the value found in controls, when testing the serum of a cow suspected of having BSE.

CLAIMS

1. A method for diagnosing spongiform disease or demyelinating disease in vertebrates, including BSE, MS and CJD, which comprises assaying a biological sample for antibodies which bind to myelin and/or myelin neurofilaments or to one or more antigenic (immunogenic) parts thereof.

2. A method according to claim 1, in which the antibodies are IgA antibodies.

3. A method according to claim 1 or 2, in which the assay is for antibodies that bind to vertebrate myelin and/or myelin neurofialaments or parts thereof .

4. A method according to claim 3, in which the vertebrate is bovine or human.

5. A method according to claim 4, in which the test antigen is a peptide selected from the group consisting of peptides having sequences identified as Sequence ID Nos. 1 to 8 hereinbefore specified.

6. A method according to any of claims 1 to 5, in which a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.

7. A method according to any of the preceding claims combined with an assay for antibodies to Acinetobacter species.

8. A diagnostic kit for the detection of spongiform disease or demyelinating disease in vertebrates comprising, as test antigen, myelin and/or myelin neurofilaments and/or one or more parts thereof.

9. A diagnostic kit according to claim 8, in which the test antigen is a peptide having a sequence selected from the group consisting of Sequence ID Nos. 1 to 8 specified hereinbefore.

10. A diagnostic kit according to claim 8 or 9, containing as test antigens myelin, myelin neurofilaments, and *Acinetobacter calcoaceticus*.

FIG. 1

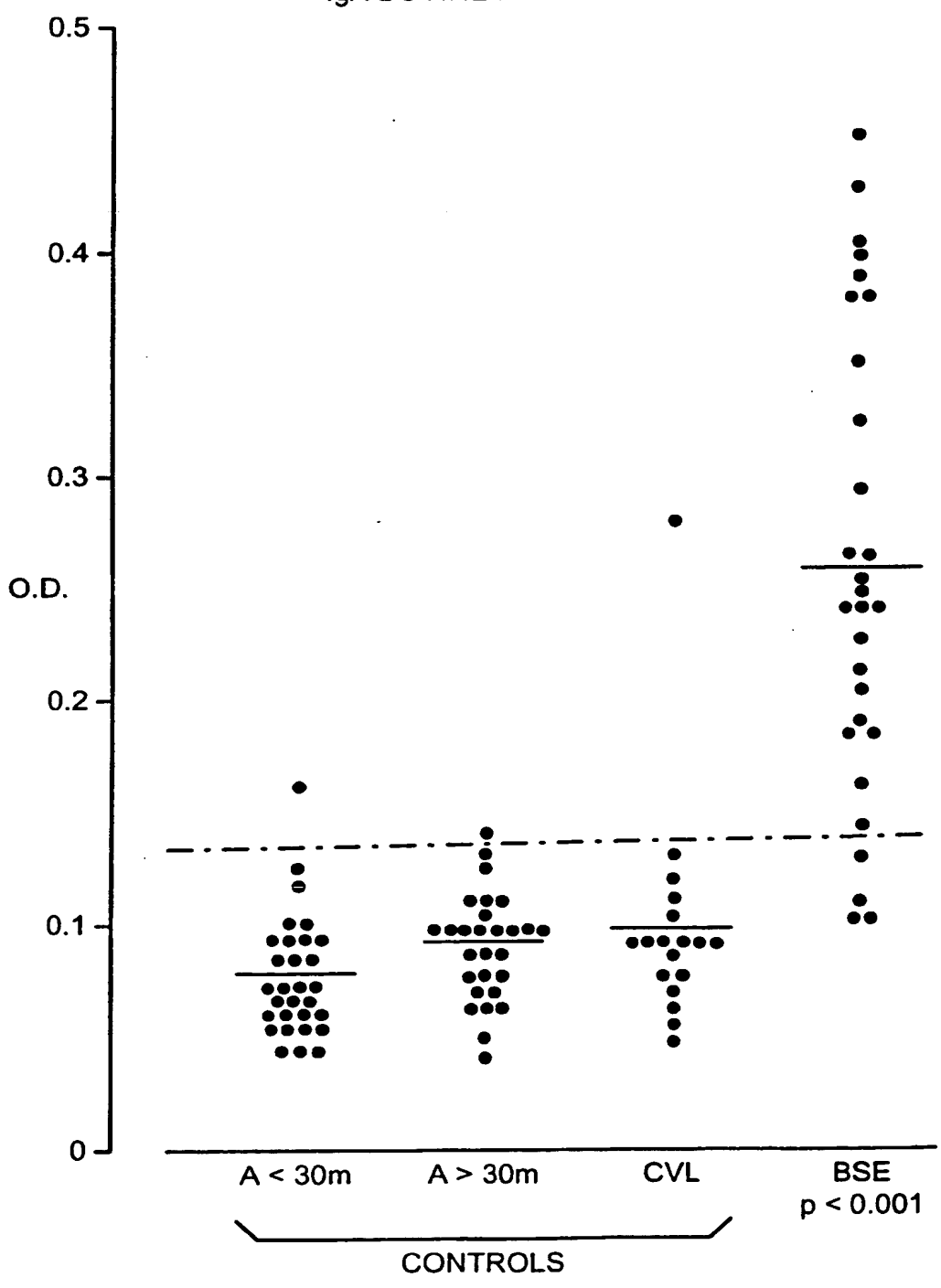

IgA BOVINE MYELIN
0.5
0.4
0.3
O.D.
0.2
0.1
0
A < 30m
A > 30m
CVL
BSE
p < 0.001
CONTROLS

FIG. 2

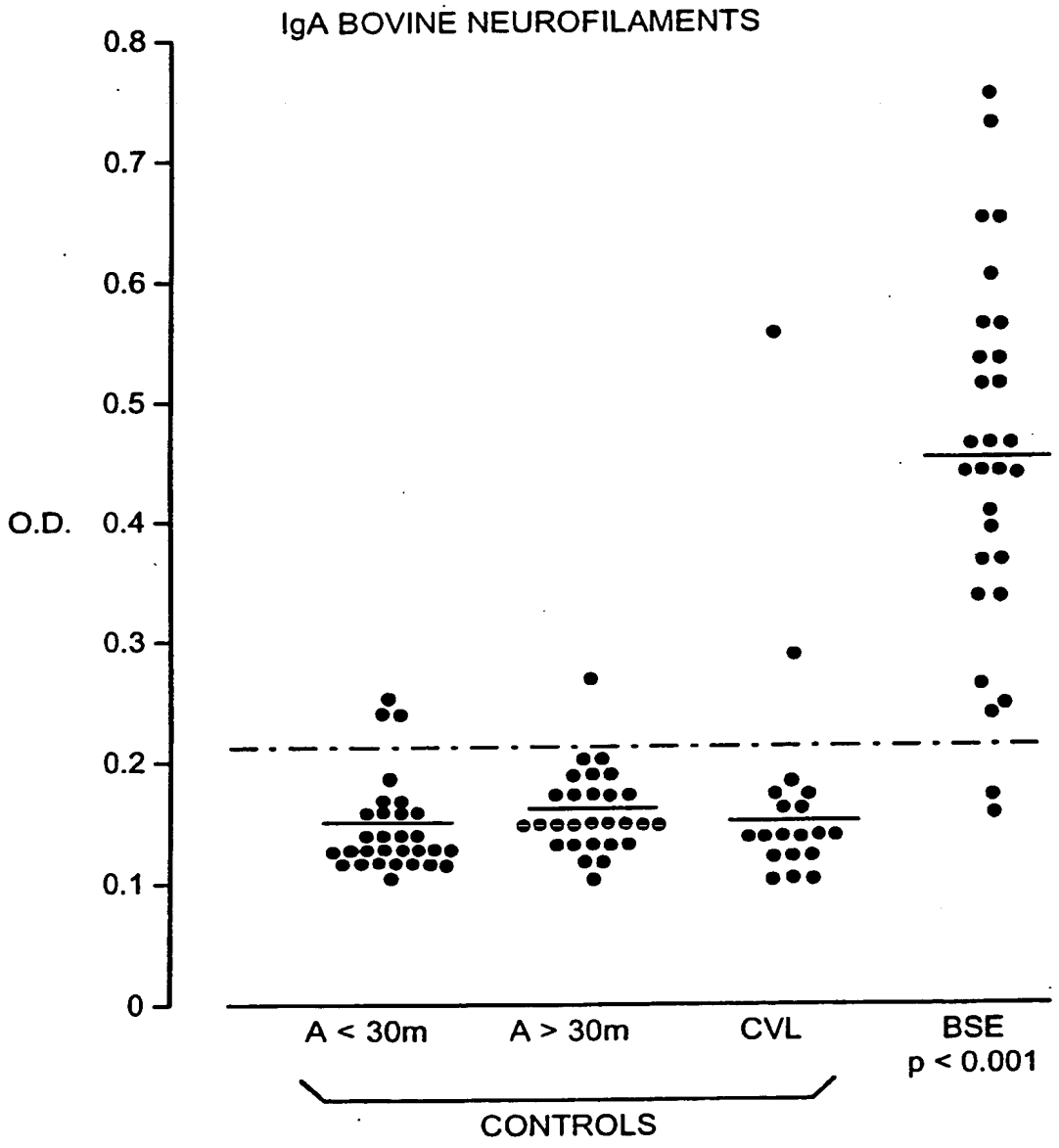

IgA BOVINE NEUROFILAMENTS
O.D.
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
A < 30m
A > 30m
CVL
BSE
p < 0.001
CONTROLS

FIG. 3

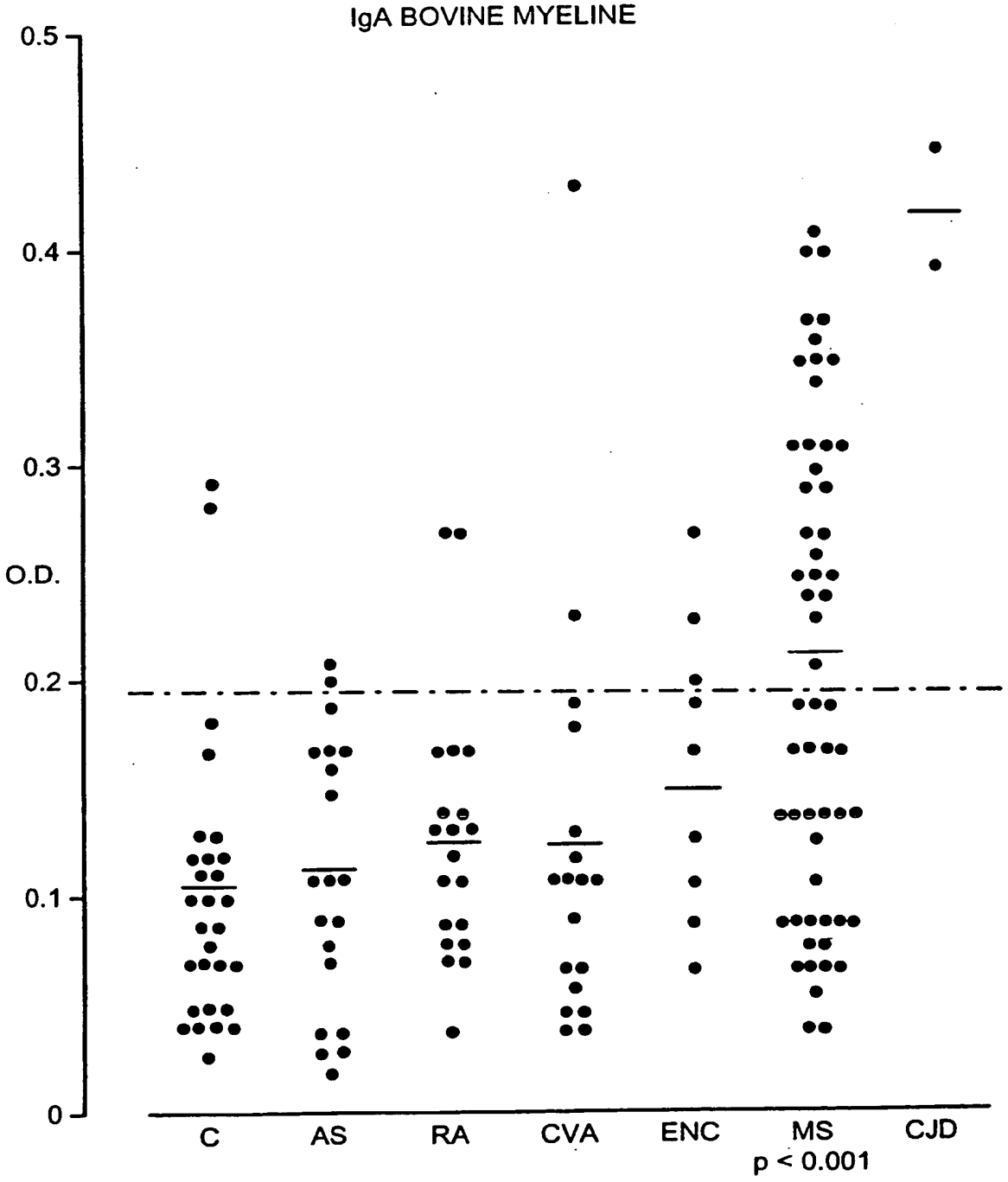
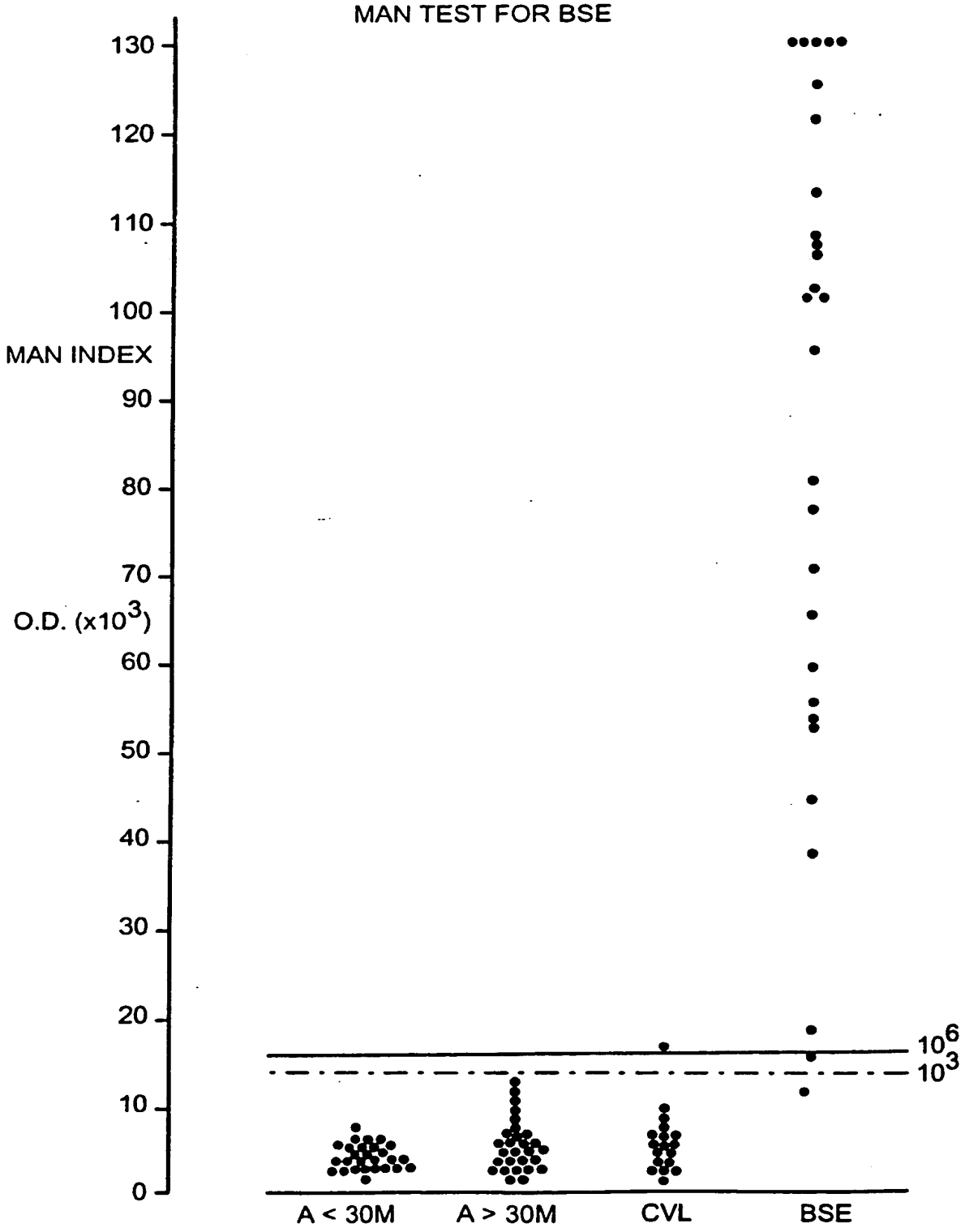

IgA BOVINE MYELINE
0.5
0.4
0.3
O.D.
0.2
0.1
0
C
AS
RA
CVA
ENC
MS
CJD
p < 0.001

FIG. 4

MAN TEST FOR BSE



INTERNATIONAL SEARCH REPORT

Intern nal Application No
PCT/GB 99/03936

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EBRINGER, A. ET AL.: "Bovine Spongiform Encephalopathy: Is It an Autoimmune Disease Due to Bacteria Showing Molecular Mimicry with Brain Antigens" ENVIRONMENTAL HEALTH PERSPECTIVES, vol. 105, no. 11, November 1997 (1997-11), pages 1172-1174, XP000892832	1,3,4,7, 8
A	table 1	2,5,9
X	WO 98 13694 A (EBRINGER ALAN ;KING S COLLEGE (GB)) 2 April 1998 (1998-04-02) cited in the application	1,3,4,7, 8
A	page 4, line 13 -page 5; claim 1	2,5,9

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 March 2000	05/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gundlach, B

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 99/03936

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A WAJGT: "Assessment by immunofluorescence methods of humoral antimyelin antibody in rats with cyanide encephalopathy" CHEMICAL ABSTRACTS + INDEXES,US,AMERICAN CHEMICAL SOCIETY. COLUMBUS, vol. 11, no. 80, 18 March 1974 (1974-03-18), page 68 XP002052988 ISSN: 0009-2258 abstract	1
P,X	WO 99 47932 A (EBRINGER ALAN ;KING S COLLEGE UNIVERSITY OF L (GB)) 23 September 1999 (1999-09-23)	1-4,6-8, 10
A	page 1-13	5,9

1

INTERNATIONAL SEARCH REPORT

information on patent family members

Intern[illegible]al Application No
PCT/GB 99/03936

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9813694 A	02-04-1998	EP 0929813 A	21-07-1999
WO 9947932 A	23-09-1999	AU 2948799 A	11-10-1999